Withdrawal of the restriction between PR33a and PR33b is requested. As explained in the response filed July 1, 2003, these sequences are highly related. A search for PR33a would necessarily overlap with PR33b.

For example, as explained in detail on Page 3-6 of the specification, SEQ ID NOS 1 and 3 are related sequences:

> PR33a (SEQ ID NO. 1) and PR33b (SEQ ID NO. 3) are structurally related sequences. PR33a is about 5217 nucleotides in length, including a polyA tail, and has two Alu-type sequences at about nucleotide positions 319-440 (Alu I) and 2010-2226 (Alu II), both in a reverse or antisense orientation. PR33b is about 5093 nucleotides in length, including a polyA tail, and has a single Alu sequence in reverse at nucleotide positions 1837-2092 which corresponds to the Alu II sequence of PR33a, but is missing the Alu I sequence. SEQ ID NO. 2 is the nucleotide sequence which is present in Pr33a, but absent from Pr33b. PR33a has an additional CAG triplet (the Alu I sequence, itself, has a 3' CAG triplet at its terminus) adjoining the 3' end of its Alu I sequence which is absent from PR33b. Other than these two differences, PR33a and PR33b share the same nucleotides sequence and appear to arise from the same gene (see below). In addition to the transcripts corresponding to PR33a and PR33b, other cDNAs arising from the same gene have been detected. These are described in more detail below in the section describing genomic DNA.

Thus, it is not logical to restrict the claims between these two nucleotide sequences.

The claims stand rejected over 35 U.S.C. §101. Attached is a post-filing publication (Exhibit B) establishing that expression of PCGEM1, a shorter and incomplete form of the PR33 family (sequences are compared in Exhibit A), is significantly higher in prostate cancer. This publication provides clear evidence of a utility of PR33, as asserted in the specification, e.g., Page 1, lines 15-30.

In view of the above remarks, favorable reconsideration is courteously requested. If there are any remaining issues which could be expedited by a telephone conference, the Examiner is courteously invited to telephone counsel at the number indicated below.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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Attorney Docket No.: ORIGEN-0034

Date: September 1, 2004

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PR33a           GAAACTTTAAAATATCCCTCAGTGCTCCTGTTAATTCATGGTAGTGCCCCAAGGCACTCT
PCGEM1          ----------------------------------------------------AAGGCACTCT
PR33b           GAAACTTTAAAATATCCCTCAGTGCTCCTGTTAATTCATGGTAGTGCCCCAAGGCACTCT
                                                                   **********

PR33a           GGCACCCAGTTTTGGAACTGCAGTTTTAAAAGTCATAAATTGAATGAAAATGATAGCAAA
PCGEM1          GGCACCCAGTTTTGGAACTGCAGTTTTAAAAGTCATAAATTGAATGAAAATGATAGCAAA
PR33b           GGCACCCAGTTTTGGAACTGCAGTTTTAAAAGTCATAAATTGAATGAAAATGATAGCAAA
                ************************************************************

PR33a           GGTGGAGGTTTTTAAAGAGCTATTTATAGGTCCCTGGACAGCATCTTTTTTCAATTAGGC
PCGEM1          GGTGGAGGTTTTTAAAGAGCTATTTATAGGTCCCTGGACAGCATCTTTTTTCAATTAGGC
PR33b           GGTGGAGGTTTTTAAAGAGCTATTTATAGGTCCCTGGACAGCATCTTTTTTCAATTAGGC
                ************************************************************

PR33a           AGCAACCTTTTTGCCCTATGCCGTAACCTGTGTCTGCAACTTCCTCTAATTGGGAAATAG
PCGEM1          AGCAACCTTTTTGCCCTATGCCGTAACCTGTGTCTGCAACTTCCTCTAATTGGGAAATAG
PR33b           AGCAACCTTTTTGCCCTATGCCGTAACCTGTGTCTGCAACTTCCTCTAATTGGGAAATAG
                ************************************************************

PR33a           TTAAGCAGATTCATAGAGCTGAATGATAAAATTGTACTACGAGATGCACTGGGACTCAAC
PCGEM1          TTAAGCAGATTCATAGAGCTGAATGATAAAATTGTACTACGAGATGCACTGGGACTCAAC
PR33b           TTAAGCAGATTCATAGAGCTGAATGATAAAATTGTACTACGAGATGCACTGGGACTCAAC
                ************************************************************

PR33a           GTGACCTTATCAAGTGAGATGGAGTCTTGCCCTGTCTCCAAGGCTGGAGCCCAATGGTGT
PCGEM1          GTGACCTTATCAAGTGAG------------------------------------------
PR33b           GTGACCTTATCAAGTGAG------------------------------------------
                ******************

PR33a           GATCTTGGCTCACTGCAACCTCCACCTCCCAGGTTCAAACGTTTCTCCTGCCTCAGCCTC
PCGEM1          ------------------------------------------------------------
PR33b           ------------------------------------------------------------

PR33a           CCAAGTAACTGGGATTACAGCAGGCTTGGTGCATTTGACACTTCATGATATCAGCCAAAG
PCGEM1          --------------------CAGGCTTGGTGCATTTGACACTTCATGATATCAGCCAAAG
PR33b           ----------------------GCTTGGTGCATTTGACACTTCATGATATCAGCCAAAG
                                       **********************************

PR33a           TGGAACTAAAAACAGCTCCTGGAAGAGGACTATGACATCATCAGGTTGGGAGTCTCCAGG
PCGEM1          TGGAACTAAAAACAGCTCCTGGAAGAGGACTATGACATCATCAGGTTGGGAGTCTCCAGG
PR33b           TGGAACTAAAAACAGCTCCTGGAAGAGGACTATGACATCATCAGGTTGGGAGTCTCCAGG
                ************************************************************

PR33a           GACAGCGGACCCTTTGGAAAAGGACTAGAAAGTGTGAAATCTATTAGTCTTCGATATGAA
PCGEM1          GACAGCGGACCCTTTGGAAAAGGACTAGAAAGTGTGAAATCTATTAGTCTTCGATATGAA
PR33b           GACAGCGGACCCTTTGGAAAAGGACTAGAAAGTGTGAAATCTATTAGTCTTCGATATGAA
                ************************************************************

PR33a           ATTCTCTGTCTCTGTAAAAGCATTTCATATTTACAAGACACAGGCCTACTCCTAGGGCAG
PCGEM1          ATTCTCTGTCTCTGTAAAAGCATTTCATATTTACAAGACACAGGCCTACTCCTAGGGCAG
PR33b           ATTCTCTGTCTCTGTAAAAGCATTTCATATTTACAAGACACAGGCCTACTCCTAGGGCAG
                ************************************************************

PR33a           CAAAAAGTGGCAACAGGCAAGCAGAGGGAAAAGAGATCATGAGGCATTTCAGAGTGCACT
PCGEM1          CAAAAAGTGGCAACAGGCAAGCAGAGGGAAAAGAGATCATGAGGCATTTCAGAGTGCACT
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PR33b       CAAAAAGTGGCAACAGGCAAGCAGAGGGAAAAGAGATCATGAGGCATTTCAGAGTGCACT
            ************************************************************

PR33a       GTCTTTTCATATATTTCTCAATGCCGTATGTTTGGTTTTATTTTGGCCAAGCATAACAAT
PCGEM1      GTCTTTTCATATATTTCTCAATGCCGTATGTTTGGTTTTATTTTGGCCAAGCATAACAAT
PR33b       GTCTTTTCATATATTTCTCAATGCCGTATGTTTGGTTTTATTTTGGCCAAGCATAACAAT
            ************************************************************

PR33a       CTGCTCAAGAAAAAAAAATCTGGAGAAAACAAAGGTGCCTTTGCCAATGTTATGTTTCTT
PCGEM1      CTGCTCAAGAAAAAAAAATCTGGAGAAAACAAAGGTGCCTTTGCCAATGTTATGTTTCTT
PR33b       CTGCTCAAGAAAAAAAAATCTGGAGAAAACAAAGGTGCCTTTGCCAATGTTATGTTTCTT
            ************************************************************

PR33a       TTTGACAAGCCCTGAGATTTCTGAGGGGAATTCACATAAATGGGATCAGGTCATTCATTT
PCGEM1      TTTGACAAGCCCTGAGATTTCTGAGGGGAATTCACATAAATGGGATCAGGTCATTCATTT
PR33b       TTTGACAAGCCCTGAGATTTCTGAGGGGAATTCACATAAATGGGATCAGGTCATTCATTT
            ************************************************************

PR33a       ACGTTGTGTGCAAATATGATTTAAAGATACAACCTTTGCAGAGAGCATGCTTTCCTAAGG
PCGEM1      ACGTTGTGTGCAAATATGATTTAAAGATACAACCTTTGCAGAGAGCATGCTTTCCTAAGG
PR33b       ACGTTGTGTGCAAATATGATTTAAAGATACAACCTTTGCAGAGAGCATGCTTTCCTAAGG
            ************************************************************

PR33a       GTAGGCACGTGGAGGACTAAGGGTAAAGCATTCTTCAAGATCAGTTAATCAAGAAAGGTG
PCGEM1      GTAGGCACGTGGAGGACTAAGGGTAAAGCATTCTTCAAGATCAGTTAATCAAGAAAGGTG
PR33b       GTAGGCACGTGGAGGACTAAGGGTAAAGCATTCTTCAAGATCAGTTAATCAAGAAAGGTG
            ************************************************************

PR33a       CTCTTTGCATTCTGAAATGCCCTTGTTGCAAATATTGGTTATATTGATTAAATTTACACT
PCGEM1      CTCTTTGCATTCTGAAATGCCCTTGTTGCAAATATTGGTTATATTGATTAAATTTACACT
PR33b       CTCTTTGCATTCTGAAATGCCCTTGTTGCAAATATTGGTTATATTGATTAAATTTACACT
            ************************************************************

PR33a       TAATGGAAACAACCTTTAACTTACAGATGAACAAACCCACAAAAGCAAAAAATCAAAAGC
PCGEM1      TAATGGAAACAACCTTTAACTTACAGATGAACAAACCCACAAAAGCAAAAAATCAAAAGC
PR33b       TAATGGAAACAACCTTTAACTTACAGATGAACAAACCCACAAAAGCAAAAAATCAAAAGC
            ************************************************************

PR33a       CCTACCTATGATTTCATATTTTCTGTGTAACTGGATTAAAGGATTCCTGCTTGCTTTTGG
PCGEM1      CCTACCTATGATTTCATATTTTCTGTGTAACTGGATTAAAGGATTCCTGCTTGCTTTTGG
PR33b       CCTACCTATGATTTCATATTTTCTGTGTAACTGGATTAAAGGATTCCTGCTTGCTTTTGG
            ************************************************************

PR33a       GCATAAATGATAATGGAATATTTCCAGGTATTGTTTAAAATGAGGGCCCATCTACAAATT
PCGEM1      GCATAAATGATAATGGAATATTTCCAGGTATTGTTTAAAATGAGGGCCCATCTACAAATT
PR33b       GCATAAATGATAATGGAATATTTCCAGGTATTGTTTAAAATGAGGGCCCATCTACAAATT
            ************************************************************

PR33a       CTTAGCAATACTTTGGATAATTCTAAAATTCAGCTGGACATTGTCTAATTGTTTTTTATA
PCGEM1      CTTAGCAATACTTTGGATAATTCTAAAATTCAGCTGGACATTGTCTAATTGTTTTTTATA
PR33b       CTTAGCAATACTTTGGATAATTCTAAAATTCAGCTGGACATTGTCTAATTGTTTTTTATA
            ************************************************************

PR33a       TACATCTTTGCTAGAATTTCAAATTTTAAGTATGTGAATTTAGTTAATTAGCTGTGCTGA
PCGEM1      TACATCTTTGCTAGAATTTCAAATTTTAAGTATGTGAATTTAGTTAATTAGCTGTGCTGA
PR33b       TACATCTTTGCTAGAATTTCAAATTTTAAGTATGTGAATTTAGTTAATTAGCTGTGCTGA
            ************************************************************
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PR33a           TCAATTCAAAAACATTACTTTCCTAAATTTTAGACTATGAAGGTCATAAATTCAACAAAT
PCGEM1          TCAATTCAAAAACATTACTTTCCTAAATTTTAGACTATGAAGGTCATAAATTCAACAAAT
PR33b           TCAATTCAAAAACATTACTTTCCTAAATTTTAGACTATGAAGGTCATAAATTCAACAAAT
                ************************************************************

PR33a           ATATCTACACATACAATTATAGATTGTTTTTCATTATAATGTCTTCATCTTAACAGAATT
PCGEM1          ATATCTACACATACAATTATAGATTGTTTTTCATTATAATGTCTTCATCTTAACAGAATT
PR33b           ATATCTACACATACAATTATAGATTGTTTTTCATTATAATGTCTTCATCTTAACAGAATT
                ************************************************************

PR33a           GTCTTTGTGATTGTTTTTAGAAAACTGAGAGTTTTAATTCATAATTACTTGATCAAAAAA
PCGEM1          GTCTTTGTGATTGTTTTTAGAAAACTGAGAGTTTTAATTCATAATTACTTGATCAAAAAA
PR33b           GTCTTTGTGATTGTTTTTAGAAAACTGAGAGTTTTAATTCATAATTACTTGATCAAAAAA
                ************************************************************

PR33a           TTGTGGGAACAATCCAGCATTAATTGTATGTGATTGTTTTTATGTACATAAGGAGTCTTA
PCGEM1          TTGTGGGAACAATCCAGCATTAATTGTATGTGATTGTTTTTATGTACATAAGGAGTCTTA
PR33b           TTGTGGGAACAATCCAGCATTAATTGTATGTGATTGTTTTTATGTACATAAGGAGTCTTA
                ************************************************************

PR33a           AGCTTGGTGCCTTGAAGTCTTTTGTACTTAGTCCCATGTTTAAAATTACTACTTTATATC
PCGEM1          AGCTTGGTGCCTTGAAGTCTTTTGTACTTAGTCCCATGTTTAAAATTACTACTTTATATC
PR33b           AGCTTGGTGCCTTGAAGTCTTTTGTACTTAGTCCCATGTTTAAAATTACTACTTTATATC
                ************************************************************

PR33a           TAAAGCATTTATGTTTTTCAATTCAATTTACATGATGCTAATTATGGCAATTATAACAAA
PCGEM1          TAAAGCATTTATGTTTTTCAATTCAATTTACATGATGCTAATTATGGCAATTATAACAAA
PR33b           TAAAGCATTTATGTTTTTCAATTCAATTTACATGATGCTAATTATGGCAATTATAACAAA
                ************************************************************

PR33a           TATTAAAGATTTCGAAATAGAATATGTGAATTGTTCACATACATAGAAATGAAAAGTTCA
PCGEM1          TATTAAAGATTTCGAAATAGAAAAAAAAAAAAAAA----------------------
PR33b           TATTAAAGATTTCGAAATAGAATATGTGAATTGTTCACATACATAGAAATGAAAAGTTCA
                ********************** *    **

PR33a           TTTCGTAAAGCAAGATGCTGGGTGAAAGAGTGCTTTTGATTGAAAGATCACTAGATTAGT
PCGEM1          ------------------------------------------------------------
PR33b           TTTCGTAAAGCAAGATGCTGGGTGAAAGAGTGCTTTTGATTGAAAGATCACTAGATTAGT

PR33a           AGAGGGCAAGACTTCTAGTCCCTAATCTACCCTTAATAGCCATGTGGTCACGTGTAAGTC
PCGEM1          ------------------------------------------------------------
PR33b           AGAGGGCAAGACTTCTAGTCCCTAATCTACCCTTAATAGCCATGTGGTCACGTGTAAGTC

PR33a           AGTGAACCCATCTCATTCTCCTCATACTTTTTTCATCTCTAAAATGAGGGTATAATTTAA
PCGEM1          ------------------------------------------------------------
PR33b           AGTGAACCCATCTCATTCTCCTCATACTTTTTTCATCTCTAAAATGAGGGTATAATTTAA

PR33a           GCTCTTCATTTTTTTTTTTTTTTGAGATAGAGTTTTGCTCTTGTCACCCAGGTTGGAGTG
PCGEM1          ------------------------------------------------------------
PR33b           GCTCTTCATTTTTTTTTTTTTTTGAGATAGAGTTTTGCTCTTGTCACCCAGGTTGGAGTG

PR33a           CAATGGCACGATCTCAGCTCACTGCAACCCTCTGCTTCCTCGGTTCAAGTGATTCTCCTG
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PCGEM1      ------------------------------------------------------------
PR33b       CAATGGCACGATCTCAGCTCACTGCAACCCTCTGCTTCCTCGGTTCAAGTGATTCTCCTG

PR33a       CTTCAGCCTCCCAAGTAGCCGGGATTACAGGTGCCCGCCACCACATCTGGCTAATTTTTT
PCGEM1      ------------------------------------------------------------
PR33b       CTTCAGCCTCCCAAGTAGCCGGGATTACAGGTGCCCGCCACCACATCTGGCTAATTTTTT

PR33a       GTATTTTCACCATGTTGGCCAGGCTGGTCTCGAACCCCTACCTCAGGTGATCCCTCGCCT
PCGEM1      ------------------------------------------------------------
PR33b       GTATTTTCACCATGTTGGCCAGGCTGGTCTCGAACCCCTACCTCAGGTGATCCCTCGCCT

PR33a       CGGCCTCTCAAAGTGCTGGGATTACAGGTGTGAGCCACCACGCCCAGCCCAATATCAGTT
PCGEM1      ------------------------------------------------------------
PR33b       CGGCCTCTCAAAGTGCTGGGATTACAGGTGTGAGCCACCACGCCCAGCCCAATATCAGTT

PR33a       TTTCTTTTTTAACACAAGGCTAACACAATCAAAATACTAGCTAGGGGAGAAAAAAAAAAT
PCGEM1      ------------------------------------------------------------
PR33b       TTTCTTTTTTAACACAAGGCTAACACAATCAAAATACTAGCTAGGGGAGAAAAAAAAAAT

PR33a       AAGGCACTGTTTATGTGTAACAGGCTCTTGTTGCAATCACTGGGCAGACAATAAACAGTA
PCGEM1      ------------------------------------------------------------
PR33b       AAGGCACTGTTTATGTGTAACAGGCTCTTGTTGCAATCACTGGGCAGACAATAAACAGTA

PR33a       AGAATCAATCCTTTTCATATATCCTTCTTGCAGAATACATAAAATCCCACAAATGGCTAT
PCGEM1      ------------------------------------------------------------
PR33b       AGAATCAATCCTTTTCATATATCCTTCTTGCAGAATACATAAAATCCCACAAATGGCTAT

PR33a       CTTCCTTTTTATGATATTTGGAGAATTGTAGCTAAGTGACAGATATTTTGCTTGGGTGTA
PCGEM1      ------------------------------------------------------------
PR33b       CTTCCTTTTTATGATATTTGGAGAATTGTAGCTAAGTGACAGATATTTTGCTTGGGTGTA

PR33a       TAGACCACAAAGGACTGTGTTTGATGATGGTTTGCATAAAATTATACCTTAGTTTTTACT
PCGEM1      ------------------------------------------------------------
PR33b       TAGACCACAAAGGACTGTGTTTGATGATGGTTTGCATAAAATTATACCTTAGTTTTTACT

PR33a       TTGTATGTTACATGTTAGATTTAGAGTATGAAAATTAGTAGGGAGGATTATTAACAAAGA
PCGEM1      ------------------------------------------------------------
PR33b       TTGTATGTTACATGTTAGATTTAGAGTATGAAAATTAGTAGGGAGGATTATTAACAAAGA

PR33a       ACAGGGCAAGAGGAGTAGAATTAAACCTCTTCTAATACCTGTGCACAAGTAGGCTTTTCA
PCGEM1      ------------------------------------------------------------
PR33b       ACAGGGCAAGAGGAGTAGAATTAAACCTCTTCTAATACCTGTGCACAAGTAGGCTTTTCA

PR33a       GAAACTCTACAACCCTACATAAACTGGATAGTTAGAAAAGCACACTCCCAAGGAAGGCGG
PCGEM1      ------------------------------------------------------------
PR33b       GAAACTCTACAACCCTACATAAACTGGATAGTTAGAAAAGCACACTCCCAAGGAAGGCGG
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PR33a           TTATGTTTTGCAGTTTGAATCAGAAGAATAGAGCTATAGCAATCTTCATTCTATAGTAAC
PCGEM1          ------------------------------------------------------------
PR33b           TTATGTTTTGCAGTTTGAATCAGAAGAATAGAGCTATAGCAATCTTCATTCTATAGTAAC

PR33a           ATTAAAGAGCCTGGTTTATATTATAGCAGTCATTAAGATTTAAAAATTTACATCTTGCCG
PCGEM1          ------------------------------------------------------------
PR33b           ATTAAAGAGCCTGGTTTATATTATAGCAGTCATTAAGATTTAAAAATTTACATCTTGCCG

PR33a           TTCTTCTTACTCACAGATTTTCGAGAGGTAATGTAATGATCCACGAGGTGAGAATCACTG
PCGEM1          ------------------------------------------------------------
PR33b           TTCTTCTTACTCACAGATTTTCGAGAGGTAATGTAATGATCCACGAGGTGAGAATCACTG

PR33a           CCTTTTATAATGCGATTAAATTGCATGAACAAAGTTTCCAACAAATAACAGTAATAAAAA
PCGEM1          ------------------------------------------------------------
PR33b           CCTTTTATAATGCGATTAAATTGCATGAACAAAGTTTCCAACAAATAACAGTAATAAAAA

PR33a           GAAACATGTATTAGCACTTAATAAGCCAGGGGCTGGACGACGTGTGTTACATGCTTTCAA
PCGEM1          ------------------------------------------------------------
PR33b           GAAACATGTATTAGCACTTAATAAGCCAGGGGCTGGACGACGTGTGTTACATGCTTTCAA

PR33a           TCCATGAACTGGTAAACTGGTACTAGTATCTCTATTGGACATGTGAGGAAACCAAATGGA
PCGEM1          ------------------------------------------------------------
PR33b           TCCATGAACTGGTAAACTGGTACTAGTATCTCTATTGGACATGTGAGGAAACCAAATGGA

PR33a           GTTGATAAACAGTAGAGTTAAAAATTACTCTTCATATATTATATTGCCTCAATCTCACAG
PCGEM1          ------------------------------------------------------------
PR33b           GTTGATAAACAGTAGAGTTAAAAATTACTCTTCATATATTATATTGCCTCAATCTCACAG

PR33a           ACATCTCTGCTACCAAAAGCTATCATATCTAGATATGCGGCATAAGGATGACCTTGGGGC
PCGEM1          ------------------------------------------------------------
PR33b           ACATCTCTGCTACCAAAAGCTATCATATCTAGATATGCGGCATAAGGATGACCTTGGGGC

PR33a           ACACTAGAATTCTTTGAGAGAATTCTGGCAGAGAAAACAAATATTTATTCCTACAATAAA
PCGEM1          ------------------------------------------------------------
PR33b           ACACTAGAATTCTTTGAGAGAATTCTGGCAGAGAAAACAAATATTTATTCCTACAATAAA

PR33a           ACCCAGCATTTTACAGGTTTTATTTTTAACTATGAAGTATTGTTATCTGTATCTTTCATA
PCGEM1          ------------------------------------------------------------
PR33b           ACCCAGCATTTTACAGGTTTTATTTTTAACTATGAAGTATTGTTATCTGTATCTTTCATA

PR33a           TAAGTGTGCCCGGAATTTATTTCTTCTGGTGGGTTCTTGGTCTCGCTGACTCCAAGAATG
PCGEM1          ------------------------------------------------------------
PR33b           TAAGTGTGCCCGGAATTTATTTCTTCTGGTGGGTTCTTGGTCTCGCTGACTCCAAGAATG
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PR33a           AAACCGCAGACCCTTGAGGTGAGTGTCACAGTTCTTAAAGATGGTGTGTTCAGAGTTTGT
PCGEM1          ------------------------------------------------------------
PR33b           AAACCGCAGACCCTTGAGGTGAGTGTCACAGTTCTTAAAGATGGTGTGTTCAGAGTTTGT

PR33a           TCCTTCAGATGTTCAGATGTGTCCGGAGTTTCTCCCTTATGGTGAGTTCGTGGTCTCGCT
PCGEM1          ------------------------------------------------------------
PR33b           TCCTTCAGATGTTCAGATGTGTCCGGAGTTTCTCCCTTATGGTGAGTTCGTGGTCTCGCT

PR33a           GACTTCAACAATGAAGCCGCAGACCTTTGCAGTGAGTGTGTGACAGTTCTTAAAGGCAGT
PCGEM1          ------------------------------------------------------------
PR33b           GACTTCAACAATGAAGCCGCAGACCTTTGCAGTGAGTGTGTGACAGTTCTTAAAGGCAGT

PR33a           GCGTCCAGAGTTGTTTGTTCCTCCCGGTAGGTTCGTGGTCTCGCTGATGTCAGGAATGAA
PCGEM1          ------------------------------------------------------------
PR33b           GCGTCCAGAGTTGTTTGTTCCTCCCGGTAGGTTCGTGGTCTCGCTGATGTCAGGAATGAA

PR33a           GCTGCAGACCCTCGCGGTAAGTGTTACAGCTCATAAAGGTAGTGCAAACCCAAACAGTGA
PCGEM1          ------------------------------------------------------------
PR33b           GCTGCAGACCCTCGCGGTAAGTGTTACAGCTCATAAAGGTAGTGCAAACCCAAACAGTGA

PR33a           GCAGTAGCAAGATTTATTATGAAGAGCAAAAGAACAAAGCTTCCCCACCATAGAAACGGA
PCGEM1          ------------------------------------------------------------
PR33b           GCAGTAGCAAGATTTATTATGAAGAGCAAAAGAACAAAGCTTCCCCACCATAGAAACGGA

PR33a           CCAGAATTGGTTGCTGCTGCTGTGGTAGCCAGCTTTTATTCCCTTATTTGGCCACACCCA
PCGEM1          ------------------------------------------------------------
PR33b           CCAGAATTGGTTGCTGCTGCTGTGGTAGCCAGCTTTTATTCCCTTATTTGGCCACACCCA

PR33a           CATCCTGCTGATTGGCCCATTTTACAGAATGCTGATTGGTCCATTTTATAGCGTGCTGAT
PCGEM1          ------------------------------------------------------------
PR33b           CATCCTGCTGATTGGCCCATTTTACAGAATGCTGATTGGTCCATTTTATAGCGTGCTGAT

PR33a           TGGTGCGTTTTTACAGAGTGCTGATTGGTGCATTTACAATCCTTTAGCTAGACACAGAGT
PCGEM1          ------------------------------------------------------------
PR33b           TGGTGCGTTTTTACAGAGTGCTGATTGGTGCATTTACAATCCTTTAGCTAGACACAGAGT

PR33a           GCTGATTGGTGCCTTTATAATCCTTTAGCTAGACACAAAAGTTCTACAAGTCCCCACCCA
PCGEM1          ------------------------------------------------------------
PR33b           GCTGATTGGTGCCTTTATAATCCTTTAGCTAGACACAAAAGTTCTACAAGTCCCCACCCA

PR33a           ACCCAGAAGCTCCGCTGGCTTCACCTCTCGTAAGGAAATTGAGGTTCAAACAAGTTTCAA
PCGEM1          ------------------------------------------------------------
PR33b           ACCCAGAAGCTCCGCTGGCTTCACCTCTCGTAAGGAAATTGAGGTTCAAACAAGTTTCAA

PR33a           AGTGCTAAAACTACAGTTTCTCATTCTCTGCAACTGGATTTCCACTCATGTGTTTGAATC
PCGEM1          ------------------------------------------------------------
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PR33b          AGTGCTAAAACTACAGTTTCTCATTCTCTGCAACTGGATTTCCACTCATGTGTTTGAATC

PR33a          CCAGGCTCTAAGACTTAACTTGCCATTCTGTGACTTTATGTTCCTGCAATTTACACAAAG
PCGEM1         ------------------------------------------------------------
PR33b          CCAGGCTCTAAGACTTAACTTGCCATTCTGTGACTTTATGTTCCTGCAATTTACACAAAG

PR33a          CTACTATCTGTCACATCTCTGGTGTTAACTTCAGACTAAACTTCTTTTTGATTCACAATG
PCGEM1         ------------------------------------------------------------
PR33b          CTACTATCTGTCACATCTCTGGTGTTAACTTCAGACTAAACTTCTTTTTGATTCACAATG

PR33a          ACCACACACTTTTTGGTTGAGGTTTTGCTATCGGTTTATTGTACTGGTTAATAGAGAGCT
PCGEM1         ------------------------------------------------------------
PR33b          ACCACACACTTTTTGGTTGAGGTTTTGCTATCGGTTTATTGTACTGGTTAATAGAGAGCT

PR33a          TCTTCCAGAAATTTGAGTAGATGGAAGAGGAAGTAGCACATTCCTAAAAATGTACCATGC
PCGEM1         ------------------------------------------------------------
PR33b          TCTTCCAGAAATTTGAGTAGATGGAAGAGGAAGTAGCACATTCCTAAAAATGTACCATGC

PR33a          CTTTCAAGTCACAAGCATCCCTATCACATGGCTGTCAAGGGTGGCTCAGAATAGGTAGAG
PCGEM1         ------------------------------------------------------------
PR33b          CTTTCAAGTCACAAGCATCCCTATCACATGGCTGTCAAGGGTGGCTCAGAATAGGTAGAG

PR33a          TTAAGAATTTAAAGTAAATTGGTGTAAGCGATGAAAGCTTCATCTAAAAGCTTATATTAC
PCGEM1         ------------------------------------------------------------
PR33b          TTAAGAATTTAAAGTAAATTGGTGTAAGCGATGAAAGCTTCATCTAAAAGCTTATATTAC

PR33a          ATCAACTGAAATGTAAAATAATTGGAACATTTTCCAGGCATCCCTGTTATTTATTTGTCT
PCGEM1         ------------------------------------------------------------
PR33b          ATCAACTGAAATGTAAAATAATTGGAACATTTTCCAGGCATCCCTGTTATTTATTTGTCT

PR33a          CTCTTTCCTTGCTTGCCTACTTCAAAAGTCATATGGCATGGTGACTAGAACTGTCCTGCC
PCGEM1         ------------------------------------------------------------
PR33b          CTCTTTCCTTGCTTGCCTACTTCAAAAGTCATATGGCATGGTGACTAGAACTGTCCTGCC

PR33a          AAAGAGTTTGTCAATATAAGATTCCTTTCTTTGTAAACATTCTACCTTGGGGCTTCATTT
PCGEM1         ------------------------------------------------------------
PR33b          AAAGAGTTTGTCAATATAAGATTCCTTTCTTTGTAAACATTCTACCTTGGGGCTTCATTT

PR33a          ATAATCAAAAGGAGTACTGTAACCTGTCAAAAAAAAGCTACCTGTGACAATATATTATGT
PCGEM1         ------------------------------------------------------------
PR33b          ATAATCAAAAGGAGTACTGTAACCTGTCAAAAAAAAGCTACCTGTGACAATATATTATGT

PR33a          GATGGTTACCTGCAGTAAGGTGGTGGCAATAAATAAATAAATAATCACAGAATGAAACCG
PCGEM1         ------------------------------------------------------------
PR33b          GATGGTTACCTGCAGTAAGGTGGTGGCAATAAATAAATAAATAATCACAGAATGAAACCG
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PR33a           AGCAGAACTGTCAGAGAAATGGTCAGAATTCACACTCTGAAGAACACGGCTATACAGTAA
PCGEM1          ------------------------------------------------------------
PR33b           AGCAGAACTGTCAGAGAAATGGTCAGAATTCACACTCTGAAGAACACGGCTATACAGTAA

PR33a           TAATCATAATAAATAGCCACTCAATCCAAAACATCACTGGGCGACTTGTCACATATATAA
PCGEM1          ------------------------------------------------------------
PR33b           TAATCATAATAAATAGCCACTCAATCCAAAACATCACTGGGCGACTTGTCACATATATAA

PR33a           TCAGTGGAGATGTGATTGAAGCACAAGGCTTAAGTGAATGTCTAGAGAGCTAATTGATTC
PCGEM1          ------------------------------------------------------------
PR33b           TCAGTGGAGATGTGATTGAAGCACAAGGCTTAAGTGAATGTCTAGAGAGCTAATTGATTC

PR33a           ATTTTTATGGAAATTTTACTTATTTTAAATGTCATCCCTGACCATCTTGAACTTTTACTT
PCGEM1          ------------------------------------------------------------
PR33b           ATTTTTATGGAAATTTTACTTATTTTAAATGTCATCCCTGACCATCTTGAACTTTTACTT

PR33a           GAAGATTTATTTTTTTTTTTAAATCACTGTTTATTAGATTTAGGTATTCTGGTCTTTGTT
PCGEM1          ------------------------------------------------------------
PR33b           GAAGATTTATTTTTTTTTTTAAATCACTGTTTATTAGATTTAGGTATTCTGGTCTTTGTT

PR33a           TTTCTTTTTTATCTATGTATGATTTTTATTTTTTTATGCAGTGTCCTTAAGCTTCATCAA
PCGEM1          ------------------------------------------------------------
PR33b           TTTCTTTTTTATCTATGTATGATTTTTATTTTTTTATGCAGTGTCCTTAAGCTTCATCAA

PR33a           TGAGAAGAAATGTATTAAAATCCATTTATTCTTACCCTAAAAAAAAAAAAAAAAAAAA
PCGEM1          ------------------------------------------------------------
PR33b           TGAGAAGAAATGTATTAAAATCCATTTATTCTTACCCTAAAAAAAAAAAAAAAAAAAA
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SHORT REPORTS

Elevated expression of *PCGEM1*, a prostate-specific gene with cell growth-promoting function, is associated with high-risk prostate cancer patients

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***PCGEM1* is a novel, highly prostate tissue-specific, androgen-regulated gene. Here, we demonstrate that *PCGEM1* expression is significantly higher in prostate cancer (CaP) cells of African-American men than in Caucasian-American men ($P = 0.0002$). Further, increased *PCGEM1* expression associates with normal prostate epithelial cells of CaP patients with a family history of CaP ($P = 0.0400$). *PCGEM1* overexpression in LNCaP and in NIH3T3 cells promotes cell proliferation and a dramatic increase in colony formation, suggesting a biological role of *PCGEM1* in cell growth regulation. Taken together, the cell proliferation/colony formation-promoting functions of *PCGEM1* and the association of its increased expression with high-risk CaP patients suggest the potential roles of *PCGEM1* in CaP onset/progression, especially in these high-risk groups.**
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Introduction

Prostate cancer (CaP) is a complex, multifactorial disease with heterogeneous tumor types, making prediction of the clinical course for individual CaP patients a difficult challenge (Small, 1998; Isaacs *et al.*, 2002). Traditional prognostic markers, such as Gleason grade, clinical stage, and pretreatment prostate-specific antigen (PSA) levels have only limited prognostic value for an individual patient (Small, 1998). The established risk factors for CaP are patient age, ethnic origin, and CaP family history (Isaacs *et al.*, 2002). The molecular mechanisms of CaP susceptibility are being currently defined (Isaacs *et al.*, 2002; Xu *et al.*, 2002).

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Among several approaches to define CaP-specific genetic alterations, comparison of global gene expression profiles in cancer cells and corresponding normal cells is emerging as a successful strategy, revealing consistent overexpression of *HEPSIN* (Dhanasekaran *et al.*, 2001; Magee *et al.*, 2001; Welsh *et al.*, 2001; Luo *et al.*, 2002) and *AMACR* (Rubin *et al.*, 2002) in CaP, and *EZH2* (Varambally *et al.*, 2002) in metastatic CaP. Recently, the expression pattern of a group of five genes was reported to correlate with CaP progression (Singh *et al.*, 2002). Gene expression alterations in tumor cells may also predispose to cancer (Yan *et al.*, 2002). CaP-specific global gene expression analyses in our laboratory have defined *PCGEM1* as a highly prostate-specific, androgen-regulated gene with cancer-associated overexpression (Srikantan *et al.*, 2000).

PCGEM1 appears to be a noncoding functional RNA gene (Srikantan *et al.*, 2000). *PCGEM1* is similar to *DD3* (Bussemakers *et al.*, 1999) in that these genes are highly prostate-specific, nonprotein-coding genes. However, no sequence homology exists between *PCGEM1* and *DD3*. Recent reviews of the literature (Szymanski and Barciszewski, 2002) and a database of noncoding RNAs demonstrate (http://biobases.ibch.poznan.pl/ncRNA/) that an increasing number of noncoding RNA genes are being discovered that may have biological functions in diverse cellular processes. *H19*, *His-1*, and *Bic* represent examples of noncoding RNAs implicated in tumorigenesis (Szymanski and Barciszewski, 2002). In this regard, *PCGEM1* and *DD3* may represent a new class of prostate-specific genes. Therefore, our laboratory is pursuing an in-depth evaluation of the biological functions of *PCGEM1* and features of its tumor-associated expression.

In order to gain an insight into the cell biologic function of *PCGEM1*, NIH3T3 and LNCaP cells overexpressing *PCGEM1* were generated. Cells were transfected with *PCGEM1* cDNA cloned into a eucaryotic expression vector (pEAK8). Puromycin-resistant transfectants were selected and pooled cell lines stably overexpressing *PCGEM1* have been established. The strong expression of *PCGEM1* RNA in both

the LNCaP and the NIH3T3 transfectants was detectable by Northern blot analysis (Figures 1a and 2a Inset). Control cells were transfected with the vector without the *PCGEM1* insert.

The effects of *PCGEM1* overexpression on cell growth/proliferation and cell cycle were evaluated. NIH 3T3 and LNCaP transfectants overexpressing *PCGEM1* were plated at low density in 96-well plates. Cell growth was followed by either counting the cell number or by a colorimetric assay. Both LNCaP cells (Figure 1a) and NIH3T3 cells (Figure 2a) overexpressing *PCGEM1* exhibited a highly significant increase in proliferation compared to the vector-control-transfected cells ($P<0.001$, calculated by *t*-test). Further, in colony-forming assays with both LNCaP cells (Figure 1b) and NIH3T3 cells (Figure 2b), overexpression of *PCGEM1* led to a dramatic increase in colony formation compared to the vector control.

The effect of *PCGEM1* overexpression on cell cycle was analysed using a panel of phosphorylation-specific antibodies raised against key cell-cycle-related proteins in Western blot experiments (CDC2, CDC25, p53, Rb Ser780, Rb Ser795, Rb Ser807/811, cyclin D1, and Chk1). In both LNCaP (Figure 1c) and NIH 3T3 (Figure 2c) cells overexpressing *PCGEM1*, a significant increase in Rb phosphorylation (Ser807/811) was detected, indicating that *PCGEM1* overexpression may affect cell proliferation through Rb phosphorylation. Rb Ser807/811 is often phosphorylated in uveal melanoma, the most common malignancy of the eye, and Rb is functionally inactivated by this phosphorylation (Brantley and Harbour, 2000). Rb Ser807/811 phosphorylation has been shown to disrupt Rb binding to the protooncogene c-abl (Knudsen and Wang, 1996). Further experiments are needed to reveal the signal transduction pathway involving *PCGEM1* and Rb Ser807/811.

The cell proliferation/colony formation-promoting effects of *PCGEM1* overexpression in the LNCaP prostate cell line suggest that it may have a functional role in cell growth regulation in human CaP cells. Sequence analysis of mouse and rat genome sequences revealed a 131 bp region of strong homology to human *PCGEM1* (83% to mouse, 85% to rat), which weakens (about 60%) with several gaps in the surrounding regions. The homology exists only at the DNA/RNA level; no possible peptides appear to be conserved between the different species. The *PCGEM1* genomic sequence homology is between corresponding regions of human chromosome 2, mouse chromosome 1, and rat chromosome 9. RNA secondary structure prediction analysis (Mfold) indicated that *PCGEM1* RNA has a lower free energy state than coding genes of the same size, or randomized versions of the *PCGEM1* sequence. Within the 1.6 kb *PCGEM1* cDNA, the first half (1–800 bp) had much lower predicted free energy (−221) than the second half (−163), indicating that the presence of a low-energy, stable secondary RNA structure is more likely in the first 800 bp region of *PCGEM1*. Mutational analysis of *PCGEM1* as well as efforts to identify intracellular molecules binding to *PCGEM1* RNA are in progress in our laboratory.

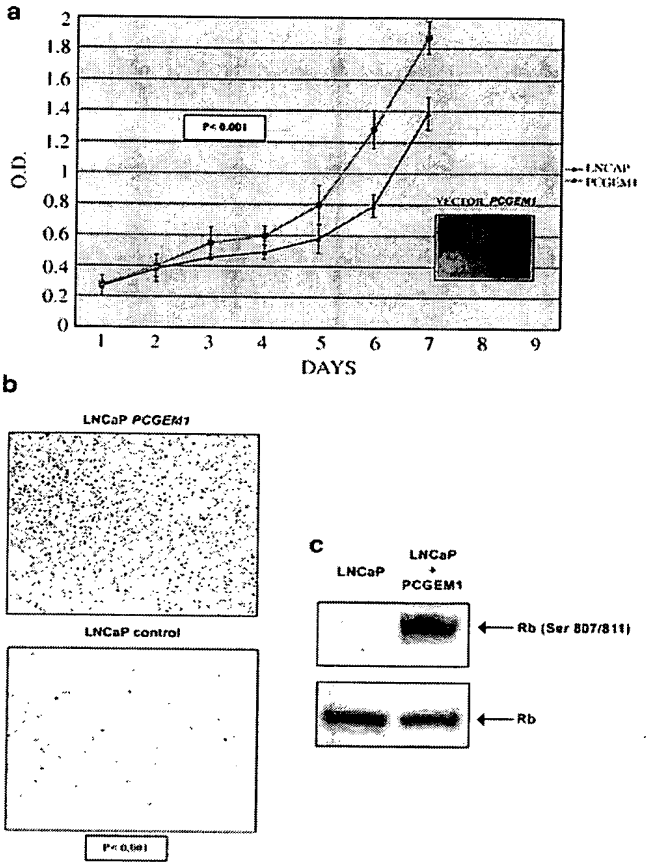

Figure 1 Increased cell proliferation and colony formation by LNCaP cells overexpressing *PCGEM1*. (**a**) Cell proliferation (plotted as optical density, measured by CellTiter Proliferation Kit, Promega) and (**b**) colony formation of LNCaP cells overexpressing *PCGEM1*. Inset: Detection of *PCGEM1* RNA by Northern blot in LNCaP *PCGEM1* transfectants. A total of 1000 cells/well were plated in 96-well plates, and cell proliferation was measured each day for 7 days in triplicate for each cell line. *P*-values for data-point average pairs were determined by the *t*-test. The $P<0.0001$ value (shown in the figure) is reached by Day 6 and Day 7. For the colony formation assay, 3000 cells were plated in 100 mm Petri dishes or T-75 cell culture flasks for each cell line. After 2 weeks, the developing individual colonies were stained with crystal violet. The number of colonies formed after 2 weeks by LNCaP parent cells (250–280) and LNCaP cells overexpressing *PCGEM1* (3000–3600) were counted in three independent experiments, and the average values were analysed by the *t*-test. The full-length *PCGEM1* cDNA was obtained in a eucaryotic expression vector (pEAK8, Edge BioSystems) by screening a prostate cDNA library (Edge BioSystems). A sequence-verified *PCGEM1* cDNA gene in the pEAK8 expression vector was transfected into both LNCaP and NIH3T3 cells using lipofectamine (Invitrogen, Carlsbad, CA, USA). Transfectants were selected by 0.2 mg/l (LNCaP) or 5 mg/l (NIH3T3) puromycin (Edge BioSystems). (**c**) Detection of Rb phosphorylation in LNCaP cells with and without *PCGEM1* expression. Protein lysates from exponentially growing cells were quantitated using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA) and 30 μg aliquots were subjected to Western blotting. Antibodies raised against phosphorylated forms of cell cycle-related proteins CDC2, CDC25, p53, Rb Ser780, Rb Ser795 and Rb Ser807/811, Chk1, as well as the Rb control antibody, were obtained from Cell Signaling Technology (Beverly, MA, USA)

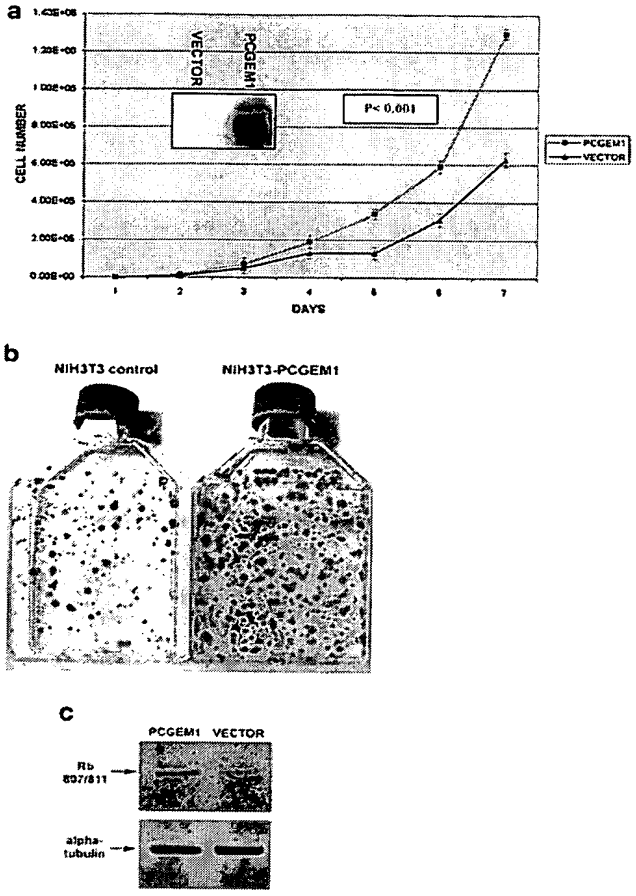

Figure 2 Cell proliferation and colony formation of NIH3T3 cells increases with *PCGEM1* overexpression. (**a**) Cell proliferation (plotted as daily cell number counts) and (**b**) colony formation of NIH3T3 cells overexpressing *PCGEM1*. Experiments were performed as described in Figure 1. Inset: Detection of *PCGEM1* RNA by Northern blot in NIH3T3 *PCGEM1* transfectants. (**c**) Detection of Rb phosphorylation in NIH3T3 cells with and without *PCGEM1* expression using the same procedure as described in Figure 1c. Alpha-tubulin antibody served as an internal control (Santa Cruz Biotechnology, Santa Cruz, CA, USA)

Although the number of noncoding RNA genes identified has sharply increased in recent years (http://biobases.ibch.poznan.pl/ncRNA/), functional data are available only on a small subset of these genes (Szymanski and Barciszewski, 2002). H19, one of the most well-characterized noncoding RNAs, exhibited tumor suppressor activity when overexpressed in embryonal carcinoma cells (Hao *et al*., 1993). Recent results, however, indicated that in breast epithelial cells, H19 overexpression promotes tumor progression without affecting cell proliferation (Lottin *et al*., 2002). His-1 (Askew *et al*., 1994) and Bic (Tam *et al*., 1997) are noncoding RNA genes whose transcription is activated by retroviral insertion, and they are implicated in the pathogenesis of hematological malignancies. Avian bic cooperates with c-*myc* in oncogenesis, and in enhancing the growth of chicken embryo fibroblasts (Tam *et al*., 2002). No functional data are reported on the prostate-specific noncoding gene DD3, which has a highly CaP-specific expression (Bussemakers *et al*., 1999).

In an attempt to assess potential clinical utility of *PCGEM1*, its expression levels in both cancer and normal prostate epithelial cells derived from radical prostatectomy specimens of CaP patients were analysed. Using OCT-embedded frozen sections of prostate tissues, prostate epithelial cells with normal and cancer phenotypes, as defined by hematoxylin–eosin (H&E) staining, were laser capture microdissected (LCM). A total of 180 RNA specimens representing microdissected paired normal and tumor cells of 90 CaP patients were quantified and used for expression analyses.

As a quality control, each LCM RNA sample was assayed by RT–PCR for the expression of NKX 3.1, a prostate epithelial cell marker (Xu *et al*., 2000). In a subset of 40 patients, the expression of *DD3*, a sensitive and specific marker of prostate tumor cells (DeKok *et al*., 2002), was also determined. Over 80% of the patients exhibited higher *DD3* expression in their prostate tumor cells than in the normal prostate epithelium (data not shown). This bank of paired normal and tumor cell-derived RNAs were screened by real-time multiplex quantitative RT–PCR (TaqMan) for the expression of *PCGEM1*. The expression data were normalized to *GAPDH* expression levels, which was measured in parallel in the same tubes (multiplex PCR).

The association of *PCGEM1* expression data with 35 different clinicopathological parameters linked to the CaP patients were analysed (full list in legends to Table 1). A summary of *PCGEM1* association with selected clinicopathological features is presented in Table 1. The statistical analyses revealed a striking association between *PCGEM1* expression levels and the ethnic origin of CaP patients. Tumor cells of African-American CaP patients ($n=22$) harbored significantly higher *PCGEM1* expression ($P=0.0002$) compared to those of Caucasian-American CaP patients ($n=66$) (Figure 3a). In contrast, *PCGEM1* expression in normal prostate epithelial cells of these two patient groups was not significantly different ($P=0.6001$) (Figure 3b). In the African-American patient population, *PCGEM1* expression was increased in their prostate tumor cells compared to matched normal prostate epithelial cells in 68.2% of the cases (15/22). On the other hand, 41% (27/66) of Caucasian-American CaP patients showed tumor cell-associated *PCGEM1* overexpression in comparison to matched normal epithelial cells. To our knowledge, this is the first observation of a prostate-specific gene with a cell growth-promoting function that shows elevated expression in African-American CaP patients, the population with the highest CaP incidence in the world. At this point, however, we cannot conclude that *PCGEM1* overexpression is cancer specific, because *PCGEM1* expression has not been evaluated in other pathological conditions of the prostate (BPH, prostatitis).

Table 1 Relationship of *PCGEM1* expression in prostate epithelial cells to clinicopathological features of CaP patients undergoing radical prostatectomy

	Tumor cells		*Normal cells*		**P-values**	
	PCGEM1 expression		*PCGEM1 expression*		*Tumor*	*Normal*
Clinicopathological features (n)	*0–3 'low'*	*4–5 'high'*	*0–3 'low'*	*4–5 'high'*		
	Patient number (n = 90)		*Patient number (n = 90)*			
Cell differentiation	58	31	61	28	0.1138	0.6158
Well (46)	32	14	31	15		
Moderate (28)	14	14	18	10		
Poor (15)	12	3	12	3		
Race	59	31	62	28	*0.0002*	0.6001
African-American (22)	7	15	14	8		
Caucasian-American (66)	51	15	46	20		
Age (years)	59	31	62	28	0.4100	0.5260
40–54 (29)	12	8	16	4		
55–59 (23)	18	5	16	7		
60–64 (27)	18	9	16	11		
65–75 (20)	11	9	14	6		
Prebiopsy PSA	58	31	61	28	0.2031	0.6705
0–4 (11)	9	2	7	4		
4.1–7 (42)	25	12	26	11		
7.1–10 (18)	13	5	14	4		
10.1+ (23)	11	12	14	9		
Family history of CaP	55	28	56	27	0.7984	*0.0400*
Yes (24)	15	9	12	12		
No (59)	40	19	44	15		
T stage	57	30	60	27	0.6016	0.6476
T2 (33)	24	9	22	11		
T3a, b (39)	24	15	26	13		
T3c (15)	9	6	12	3		
Margins	58	31	61	28	0.8174	0.1468
Neg (59)	39	20	37	22		
Pos (30)	19	11	24	6		
Seminal vesicle	58	31	61	28	0.3745	0.3723
Neg (74)	50	24	49	25		
Pos (15)	8	7	12	3		

RNA isolated (MicroRNA kit, Stratagene, La Jolla, CA, USA) from LCM samples of tumor and normal epithelial cells of radical prostatectomy patients were quantitated (RiboGreen dye, Molecular Probes, Eugene, OR and VersaFluor fluorimeter, BioRad, Hercules, CA, USA) and assayed by real-time quantitative RT–PCR (TaqMan). In all, 1 ng LCM RNA was used to produce cDNA, sufficient for 10 PCR reactions. Real-time quantitative PCR analysis was performed using TaqMan detection chemistry on the ABI Prism 7700 Sequence Detection System according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). PCR primers were: forward primer, 5′-TTCAATTAGGCAG CAACCTTT-3′; reverse primer, 5′-CATTCAGCTCTATGAATCTGCTTAA-3′; and Taqman probe, FAM-CCGTAACCTGTGTCTG CAACTTCCTCTAATT-TAMRA. Each PCR was multiplexed using the GAPDH detection control mix (Applied Biosystems, Foster City, CA, USA) as an internal control in the same tube with the *PCGEM1* mix. GAPDH control cycle threshold (C_T) values obtained from the real-time PCR assays were subtracted from the *PCGEM1* C_T values. The resulting values represent *PCGEM1* expression levels normalized to GAPDH (dC_T values). Normalized *PCGEM1* expression levels were categorized as 1 ($dC_T > 10$), 2 ($6 < dC_T < 10$), 3 ($3 < dC_T < 6$), 4 ($1 < dC_T < 3$), 5 ($dC_T < 1$), and 0 (no detectable *PCGEM1* expression). Statistical analysis was performed with the SAS software package (SAS Institute Inc., Cary, NC, USA), comparing *PCGEM1* expression categories 0–3 ('low' and no *PCGEM1* expression) to categories 4–5 ('high' *PCGEM1* expression). The association between *PCGEM1* expression and clinicopathological features was analysed using Fisher's exact test. The number of patients is in brackets. $P < 0.05$ was considered statistically significant. The prostate tissue specimens were obtained under an IRB-approved protocol from patients treated at Walter Reed Army Medical Center (WRAMC) and Uniformed Services University of the Health Sciences (USUHS). The patient database included the following clinicopathological parameters: race, prebiopsy PSA, diagnosis date, surgery date, age at surgery, left Gleason sum, right Gleason sum, worst Gleason sum, prostatitis at diagnosis, PIN, HGPIN, pretreatment testosterone, family history of CaP, T stage, neoadjuvant date, margins, tumor number; PSA recurrence: months after surgery, date, prostatitis pathology; bone metastasis: months after surgery, date; follow-up: months after surgery, latest contact date, capsule, seminal vesicle, nodes, worst grade, bladder neck, multifocal, diagnosis PSA, worst nuclear grade, prostate weight, hormone refractory date, differentiation at LCM microenvironment

Genetic, hormonal, and environmental factors may contribute to a higher risk of CaP in African-American men (Brawley *et al.*, 1998; Powell, 1998; Cussenot and Valeri, 2001). Polymorphism of genes, for example, CYP3A4 (Paris *et al.*, 1999), androgen receptor (Platz *et al.*, 2000), and vitamin D receptor (Taylor *et al.*,

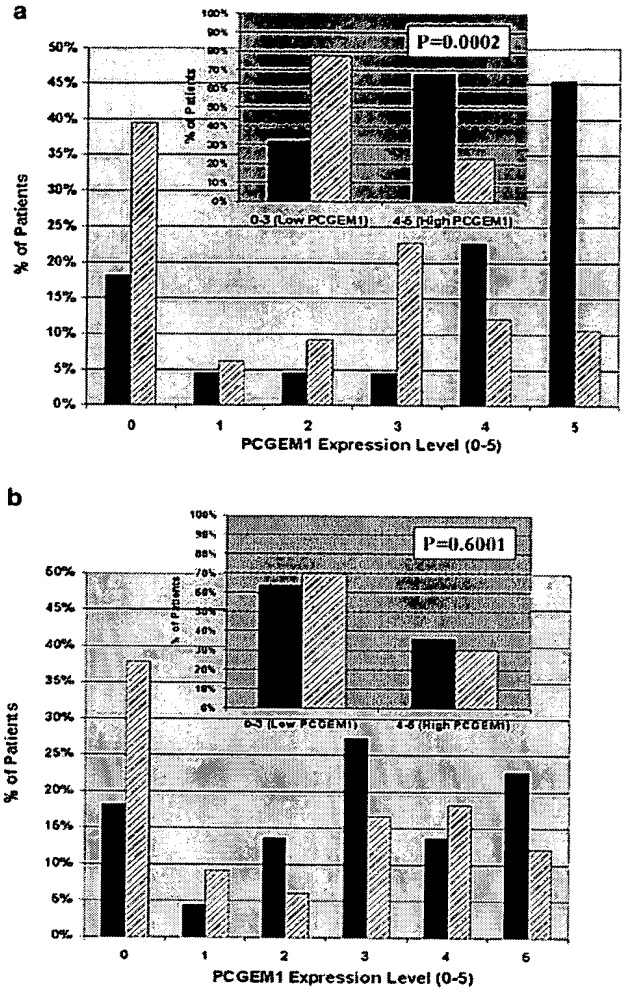

Figure 3 *PCGEM1* expression in microdissected tumor (**a**) and normal (**b**) prostate epithelial cells of African-American and Caucasian-American CaP patients. *PCGEM1* expression data were obtained and analysed as described in Table 1. The percentage of African-American (black columns) and Caucasian-American (striped columns) CaP patients expressing *PCGEM1* at different levels (0–5) in their prostate epithelial cells are represented by the columns. The *P*-value (in box) was calculated by Fisher's exact test

1996), may predispose African-American men to higher CaP risk in comparison to Caucasian-Americans. Bcl-2, an antiapoptotic protein (Guo *et al*., 2000) and caveolin-1, a membrane protein with tumor suppressor activity (Yang *et al*., 2000), show differential expression in CaP cells of African-American men compared to Caucasian-Americans. *PCGEM1* belongs to this latter category, with several interesting features: it is a prostate-specific gene, it promotes cell proliferation/colony formation, and it shows highly significant prostate tumor cell-specific overexpression in African-Americans versus Caucasian-Americans.

It is worth noting that increased PSA levels in the serum of African-American men compared to that of Caucasian-Americans have been described (Moul *et al*., 1995). Although *PCGEM1* is an androgen-regulated gene, we found no significant association between pretreatment PSA levels and *PCGEM1* expression in either tumor ($P=0.2031$) or normal ($P=0.6705$) prostate epithelial cells (Table 1), making it unlikely that a general induction of the androgen pathway would be responsible for the elevated *PCGEM1* RNA levels.

Intriguingly, our analysis of the relative levels of *PCGEM1* in histologically normal cells of CaP patients revealed a significant increase in *PCGEM1* expression in patients with a family history of CaP (12/24), as

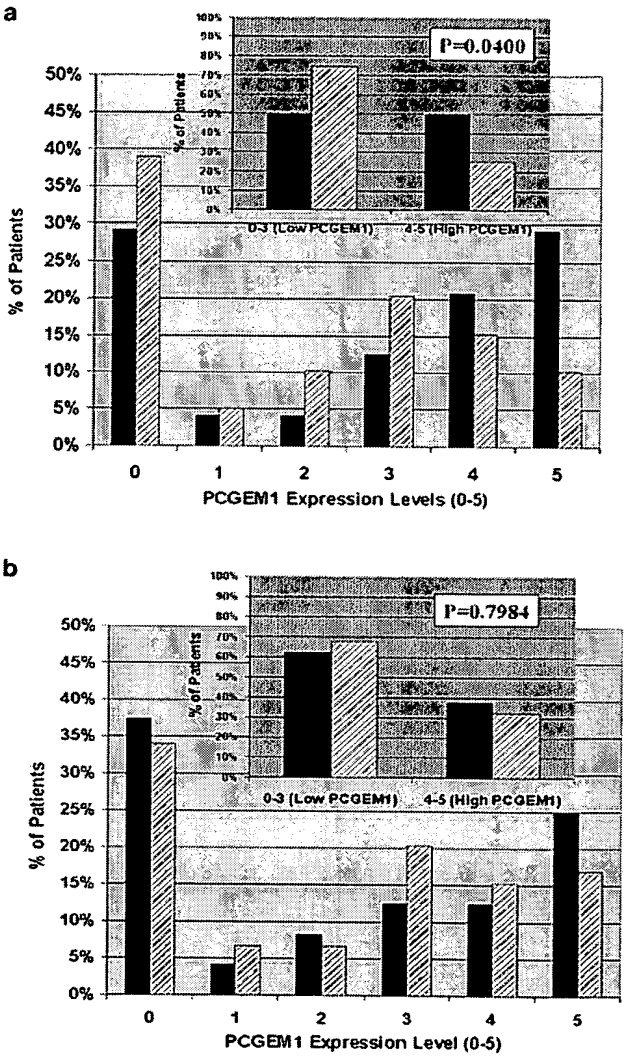

Figure 4 *PCGEM1* expression in microdissected tumor (**a**) and normal (**b**) prostate epithelial cells of CaP patients with and without family history of CaP. *PCGEM1* expression data were obtained and analysed as described in Table 1. The percentage of patients with (black columns) and without (striped columns) a family history of CaP expressing *PCGEM1* at different levels (0–5) in their prostate epithelial cells are represented by the columns. The *P*-value (in box) was calculated by Fisher's exact test

opposed to patients with no family history (15/59) ($P = 0.0400$) (Figure 4).

A multivariable logistic regression analysis with backward elimination of insignificant variables was performed for the association of *PCGEM1* expression with patient characteristics (race, family history of CaP, margin, prebiopsy PSA, prostate weight, worst Gleason sum, differentiation). The analysis revealed that the African-American race is significantly related to *PCGEM1* expression in tumor cells ($P = 0.0007$), and a family history of CaP is significantly related to *PCGEM1* expression in normal prostate epithelial cells ($P = 0.0374$). All other variables analysed were insignificant.

Potential CaP biomarkers that are consistently overexpressed in CaP cells, for example, *HEPSIN*, *AMACR*, *DD3*, and *PSMA*, do not show any reported association with ethnicity or family history of CaP (Isaacs *et al.*, 2002). Loss of *GSTP1* expression is also among the most common alterations in CaP cells (Nelson *et al.*, 2001). *PCGEM1* appears to be a distinct prostate-specific gene associated with these high-risk CaP patients. Only a few studies on cancer-associated expression of other noncoding RNA genes have been reported. H19 appears to be lost in certain embryonal tumors (Hao *et al.*, 1993), but overexpressed in a small subset of breast cancers (Lottin *et al.*, 2002). Retroviral insertion events appear to activate the expression of Bic (Tam *et al.*, 1997) and of His-1 (Askew *et al.*, 1994), but cancer-associated expression of these genes in humans has not been reported. The expanding universe of noncoding RNAs and their functional evaluations may define additional genes with potential functions in the process of tumorigenesis.

Taken together, the cell proliferation/colony formation-promoting function of a novel prostate-specific noncoding gene, *PCGEM1*, and the association of its increased expression level with high-risk CaP patients suggest the potential roles of *PCGEM1* in CaP biology. Further, *PCGEM1* expression characteristics may provide a promising biomarker and potential therapeutic target in the high-risk CaP patients noted herein.

Abbreviations

CaP, prostate cancer; PSA, prostate-specific antigen; FBS, fetal bovine serum; LCM, laser capture microdissection; RT, reverse transcription; H&E, hematoxylin–eosin.

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1: AF223389. Homo sapiens PCGE...[gi:11066459]

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SOURCE      Homo sapiens (human)
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  AUTHORS   Srikantan,V., Zou,Z., Petrovics,G., Xu,L., Augustus,M., Davis,L.,
            Livezey,J.R., Connell,T., Sesterhenn,I.A., Yoshino,K., Buzard,G.S.,
            Mostofi,F.K., McLeod,D.G., Moul,J.W. and Srivastava,S.
  TITLE     PCGEM1, a prostate-specific gene, is overexpressed in prostate
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  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 97 (22), 12216-12221 (2000)
  MEDLINE   20504490
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  AUTHORS   Srikantan,V., Zou,Z., Xu,L., Petrovics,G., Augustus,M., Davis,L.,
            Livezey,J.R., Connell,T., Sesterhenn,I.A., Yoshino,K., Buzard,G.S.,
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